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EPIDEMIOLOGY

Evidence of Sharing of *Klebsiella pneumoniae* Strains between Healthy Companion Animals and Cohabiting Humans

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ABSTRACT This study aimed to characterize the fecal colonization and sharing of *Klebsiella pneumoniae* strains between companion animals and humans living in close contact. Fecal samples were collected from 50 healthy participants (24 humans, 18 dogs, and 8 cats) belonging to 18 households. Samples were plated onto MacConkey agar (MCK) plates with and without cefotaxime or meropenem supplementation. Up to five *K. pneumoniae* colonies per participant were compared by pulsed-field gel electrophoresis (PFGE) after XbaI restriction. *K. pneumoniae* strains with unique pulse types from each participant were characterized for antimicrobial susceptibility, virulence genes, and multilocus sequence type (MLST). Fecal *K. pneumoniae* pulse types were compared to those of clinical *K. pneumoniae* strains from animal and human patients with urinary tract infections ($n = 104$). *K. pneumoniae* colonization was detected in nonsupplemented MCK in around 38% of dogs ($n = 7$) and humans ($n = 9$). *K. pneumoniae* strains isolated from dogs belonged to sequence type 17 (ST17), ST188, ST252, ST281, ST423, ST1093, ST1241, ST3398, and ST3399. None of the *K. pneumoniae* strains were multidrug resistant or hypervirulent. Two households included multiple colonized participants. Notably, two colonized dogs within household 15 (H15) shared a strain each (ST252 and ST1241) with one coliving human. One dog from H16 shared one PFGE-undistinguishable *K. pneumoniae* ST17 strain with two humans from different households; however, the antimicrobial susceptibility phenotypes of these three strains differed. Two main virulence genotypes were detected, namely *fimH-1 mrkD ycfM entB kfu* and *fimH-1 mrkD ycfM entB kpn*. These results highlight the potential role of dogs as a reservoir of *K. pneumoniae* to humans and vice versa. Furthermore, to our best knowledge, this is the first report of healthy humans and dogs sharing *K. pneumoniae* strains that were undistinguishable by PFGE/MLST.

KEYWORDS *Klebsiella pneumoniae*, animal-human sharing, clonal relatedness, companion animals, dog, humans

Klebsiella pneumoniae is an important nosocomial agent that is known to spread easily (1, 2). *K. pneumoniae* can also cause community-onset infections in companion animals and humans and is the second most common *Enterobacteriaceae* species causing urinary tract infections (UTI) in humans (3–5).

Extended spectrum beta-lactamase (ESBL) and carbapenemase-producing *Enterobacteriaceae* are frequently multidrug resistant, which leads to important therapeutic limitations (5, 6). ESBL/carbapenemase-producing *K. pneumoniae* strains are frequently reported worldwide, and their dissemination is of great importance (4, 6).

Companion animals may become infected with *K. pneumoniae* high-risk clonal

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lineages, such as ST15, which is frequently a CTX-M-15 producer (4, 7–9). However, little is known about the role of healthy dogs and cats as reservoirs of such clonal lineages.

Gut colonization by *K. pneumoniae* is strongly linked to subsequent extraintestinal infections in hospitalized human patients (1). Moreover, *Enterobacteriaceae* species that cause UTI, such as *K. pneumoniae*, are frequently part of the host gut microbiota (1). The gut of companion animals may be colonized by high-risk clonal lineages of *Escherichia coli* (10) and *Enterococcus faecium* (11), thus potentially acting as a reservoir to humans. In humans, most studies on *K. pneumoniae* fecal colonization are focused on hospitalized and/or infected patients, and therefore less information is available regarding healthy humans (1, 2). Studies on the population structure of *K. pneumoniae* strains colonizing healthy dogs and cats are also lacking.

The transmission of pathogenic bacteria from companion animals to humans has been a growing matter of concern (12). The close contact between companion animals and humans in modern society leads to greater chances of interspecies transmission of bacteria (12), including through fecal contamination. Previous studies focused on *E. coli* transmission dynamics have reported that the index strains from humans or dogs with UTI are extensively shared with other human and dog household members (13–14). Sharing of *E. coli* between healthy humans and dogs living in close contact has also been described (15–18). To our knowledge, studies on animal-human sharing have not been conducted regarding *K. pneumoniae*. However, this information is crucial to a better understanding of the epidemiology of this important pathogen.

In the current study, several households composed of healthy companion animals (dogs and cats) and humans living in close contact were enrolled to evaluate the frequency of *K. pneumoniae* colonization and animal-human sharing. Additionally, this study aimed to characterize the population structure, antimicrobial resistance, and virulence of fecal *K. pneumoniae* to aid the understanding of the role of healthy companion animals as reservoirs to humans and vice versa. A special focus regarding UTI was given by comparing the fecal *K. pneumoniae* strains with a previously characterized collection of clinical *K. pneumoniae* isolates from humans, dogs, and cats with UTI (9).

MATERIALS AND METHODS

Study population. The study population included households with at least one human and one companion animal (dog or cat) living in close contact. Prior to inclusion, all human participants were informed of the main goals of the study and were asked to sign a consent form. Participants were considered "healthy" and were included in the study if no bacterial infection nor antimicrobial use was reported in the previous month. To ensure that inclusion was anonymous, households, humans, and animals were coded with the numbered letters H, Hu, and A, respectively.

A total of 50 participants (24 humans, 18 dogs and 8 cats) living in 18 households were enrolled in 2016. Therefore, the household composition varied in the number of humans and companion animals (Fig. 1). Human participants had lived in the same household as the included companion animals for at least 6 months, except for one cat that had been recently adopted to H16 (Fig. 1).

All human participants were more than 18 years of age, and 70.8% ($n = 17/24$) were women.

Companion animal ages ranged from 2 months to 17 years, and 57.7% ($n = 15/26$) were females. Thirty-three percent ($n = 8/24$) of humans and 19.2% ($n = 5/26$) of companion animals had undergone antimicrobial treatment within the previous year. All cats lived exclusively indoors except one. All dogs had access to the outdoors; 83.3% ($n = 15/18$) lived indoors with the owners, while 16.7% ($n = 3/18$) stayed in private yards.

Sample collection and bacteriological methods. Ethical approval for this study was obtained from the Comissão de Ética e Bem-Estar Animal (CEBEA) from the Faculty of Veterinary Medicine of the University of Lisbon. All fecal samples were collected using noninvasive methods after informed, written consent was obtained. Enrolled humans collected their own fecal samples and the fecal samples from the coliving companion animals into sterile containers. Immediately after collection, fecal samples were stored at 4°C until processing.

One gram of homogenized fecal sample was added to 10 ml of sterile 0.85% NaCl (Merck, Germany) solution and mixed thoroughly. Ten microliters of fecal suspension were plated onto MacConkey agar plates (Scharlau, Spain), with or without 1.5 μg/ml of cefotaxime (Sigma-Aldrich, USA) or meropenem (Sigma-Aldrich, USA) supplementation. To improve detection of low numbers of *K. pneumoniae*, 1 g of feces was added to 5 ml of sterile buffered peptone water (Biokar Diagnostics, France), vortexed, and incubated at 36 $\pm$ 1°C for 18 h. A negative quality control consisting of buffered peptone water alone was also incubated. Following incubation, 1 μl of buffered peptone water fecal suspension was plated

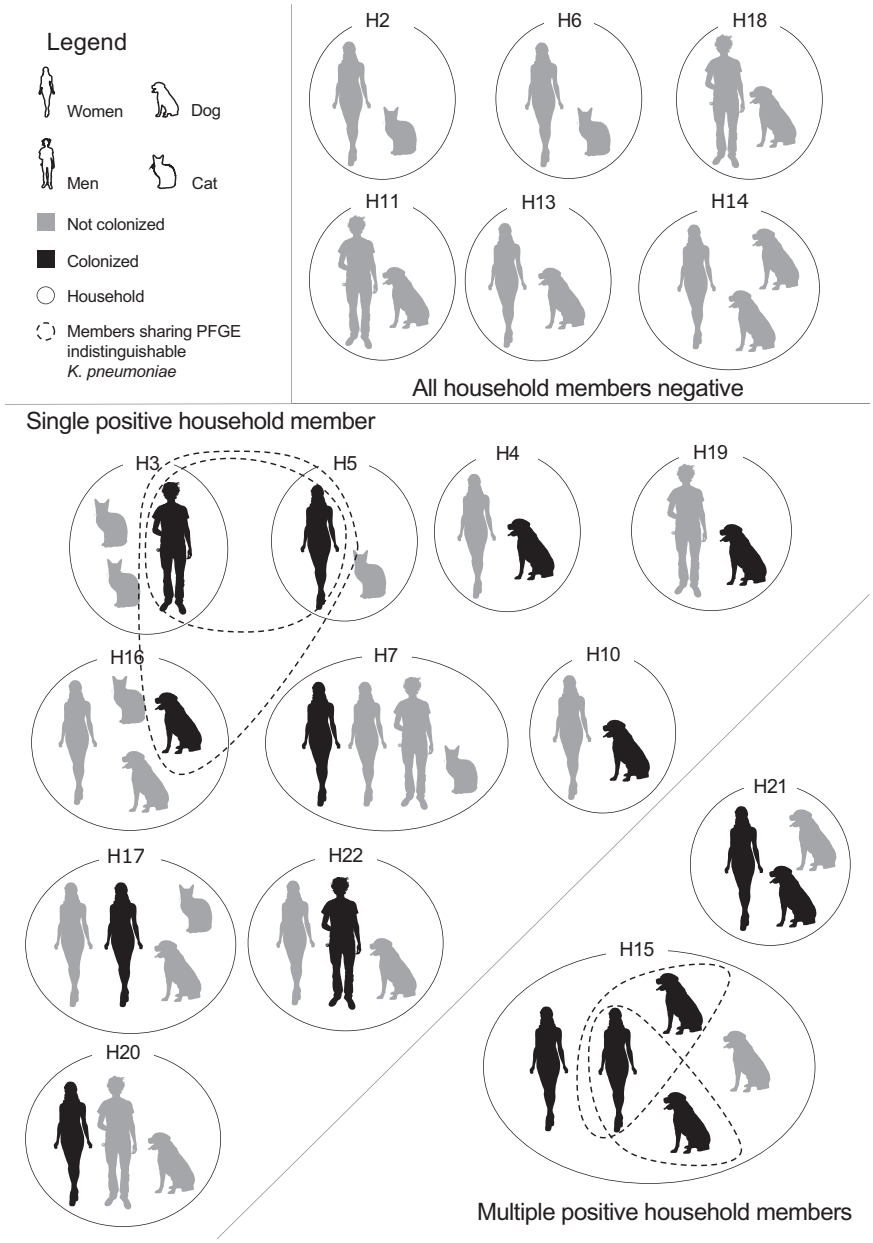


FIG 1 Fecal colonization and sharing of *K. pneumoniae* among household members.

onto the MacConkey agar plates described above. MacConkey agar plates were incubated at 36 ± 1°C for 18 h, followed by inspection for *K. pneumoniae* suspected colonies.

To guide presumptive *K. pneumoniae* identification, suspected colonies obtained from MacConkey agar plates were streaked onto UriSelect agar plates (Bio-Rad, USA). Up to five *K. pneumoniae* suspected colonies per participant were isolated and stored in 20% glycerol (Sigma-Aldrich, USA) brain heart infusion broth (Biokar Diagnostics, France) at −20°C until processing. Total DNA was extracted by the boiling method and *K. pneumoniae* species confirmed by PCR as previously described (19, 20).

All fecal samples had a high number of CFU of *Enterobacteriaceae* after direct plating onto MacConkey agar plates, thus confirming sample viability.

***K. pneumoniae* population structure analysis.** All *K. pneumoniae* isolates were compared by pulsed-field gel electrophoresis (PFGE) after 3 h XbaI (New England Biolabs, USA) restriction. Restriction fragments were separated on a CHEF-DR II apparatus (Bio-Rad, USA) using a 1% agarose gel (agarose pulse-field grade; NZYtech–Genes and Enzymes, Portugal) and previously described electrophoresis conditions (5 to 20 s for 4 h followed by 25 to 50 s for 18 h at 14°C, 6 V/cm²) (21).

K. pneumoniae strains with unique pulse types from each animal or human were further typed by MLST according to the published consensus MLST scheme (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html).

Collection of clinical UTI *K. pneumoniae* isolates. The fecal *K. pneumoniae* PFGE restriction patterns from this study were compared with those of a previously typed collection of clinical *K. pneumoniae* strains isolated from dogs and cats ($n = 27$) and from humans ($n = 77$ [19 hospital patients and 58 community patients]) with UTI (9).

Susceptibility testing. *K. pneumoniae* strains with unique pulse types from each animal and human were tested for antimicrobial resistance to amoxicillin/clavulanate 30 μg, cefoxitin 30 μg, cefotaxime 30 μg, meropenem 10 μg, ciprofloxacin 5 μg, gentamicin 10 μg, amikacin 30 μg, nitrofurantoin 300 μg, chloramphenicol 30 μg, tetracycline 30 μg, and trimethoprim-sulfamethoxazole 25 μg (Oxoid, Hampshire, UK). Antimicrobial susceptibility was conducted by disk diffusion according to CLSI guidelines (22).

Antimicrobial resistance determinants. *K. pneumoniae* isolates that were not susceptible to at least one of the tested beta-lactams, except ampicillin, were tested by PCR for the presence of bla_{TEM}, bla_{SHV}, bla_{OXA}, $bla_{CTX\text{-}M}$-type, bla_{CIT}-type, bla_{DHA}-type, bla_{MOX}-type, bla_{ACT}-type, bla_{FOX}-type, and bla_{MIR}-type beta-lactamase genes using previously described primers (23–25). *K. pneumoniae* strains with unique pulse types were tested for the presence of efflux pump (*oqxAB*) (26) and outer membrane protein (*ompK35* and *ompK36*) (27) coding genes.

Virulence genes. The *K. pneumoniae* strains that were tested for antimicrobial susceptibility were also screened by PCR for the presence of the following virulence genes: type 1 (*fimH-1*), type 3 (*mrkD*), and FimH-like (*kpn*) fimbriae adhesins (28, 29), outer membrane lipoprotein (*ycfM*) (28), catecholate siderophore receptor (*iroN*) (30), enterobactin (*entB*) (29), aerobactin (*iutA*) (29), iron transporter with phosphotransferase function (*kfu*) (29), yersiniabactin high-pathogenicity island (YHPI; *irp-1*, *irp-2*, *fyuA*, and *ybtS*) (29, 31, 32), serum resistance-associated outer membrane lipoprotein (*traT*) (31), regulator of mucoid phenotype A (*rmpA*) (29), and allantoin metabolism-associated gene (*allS*) (29).

Data analysis. *K. pneumoniae* PFGE patterns were compared using BioNumerics software version 6.6, (bioMérieux, France) and the Dice/unweighted pair group method with arithmetic mean (UMPGA) clustering method with a tolerance of 1.5% and a clustering cutoff of 80%. Previously proposed criteria for bacterial strain typing were used (33).

Fisher's exact test was used for comparisons between groups, with an alpha value of 0.05, using SAS statistical software package for Windows version 9.3 (SAS Institute, Inc., Cary, NC, USA).

K. pneumoniae STs from this study were compared with all known STs from the Institut Pasteur *K. pneumoniae* database (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html) through eBURST$_{v3}$ analysis (http://eburst.mlst.net/). *K. pneumoniae* STs were assigned to the same group if they shared identical alleles at 6 out of 7 loci with at least one other ST.

RESULTS

K. pneumoniae colonization was detected in 16 participants (7 dogs and 9 humans) from 12 households (Fig. 1). All samples were negative for *K. pneumoniae* growth in MacConkey agar plates with cefotaxime or meropenem supplementation.

The fecal colonization by *K. pneumoniae* was equally high in dogs (38.9%, $n = 7/18$) and in humans (37.5%, $n = 9/24$). However, *K. pneumoniae* colonization was not detected in cats despite the use of preenrichment prior to plating.

The majority of positive households (83.3%, $n = 10/12$) had a single colonized participant, and therefore within-household sharing of *K. pneumoniae* was absent in these cases (Fig. 1). The two households with multiple colonized participants included colonized humans and dogs (Fig. 1).

A total of 59 *K. pneumoniae* isolates were obtained from the 16 positive participants and the number of isolates per participant ranged from one to five. The PFGE analysis revealed a total of 22 different restriction patterns (A to U) (Fig. 2). In samples from which two to five *K. pneumoniae* colonies were isolated, 61.5% ($n = 8/13$) of the participants were colonized by two or more different *K. pneumoniae* strains according to the PFGE results (Fig. 2, Table 1). The PFGE analysis of the *K. pneumoniae* isolates from the participants living in household H21 showed that the human and dog were colonized by unrelated *K. pneumoniae* strains (pulse types R and H, respectively) (Fig. 2). In household H15, which was composed of two colonized humans and two colonized dogs, it is interesting to notice that while both humans were colonized by unrelated *K. pneumoniae* strains, the human H15Hu2 shared one *K. pneumoniae* strain undistinguishable by PFGE with dog H15A1 (pulse type C.1) and another with dog H15A2 (pulse type O) (Fig. 2). In both dogs from household H15, the colonizing *K. pneumoniae* strain was detected without preenrichment, while in the human H15Hu2 preenrichment was needed.

Two *K. pneumoniae* pulse types, namely E and B, were shared between humans living in distinct households (Fig. 1 and 2). *K. pneumoniae* pulse types E and B were shared by one human from household H3 and another from household H5. Furthermore, *K. pneumoniae* pulse type B was also shared by dog H16A1 from household H16

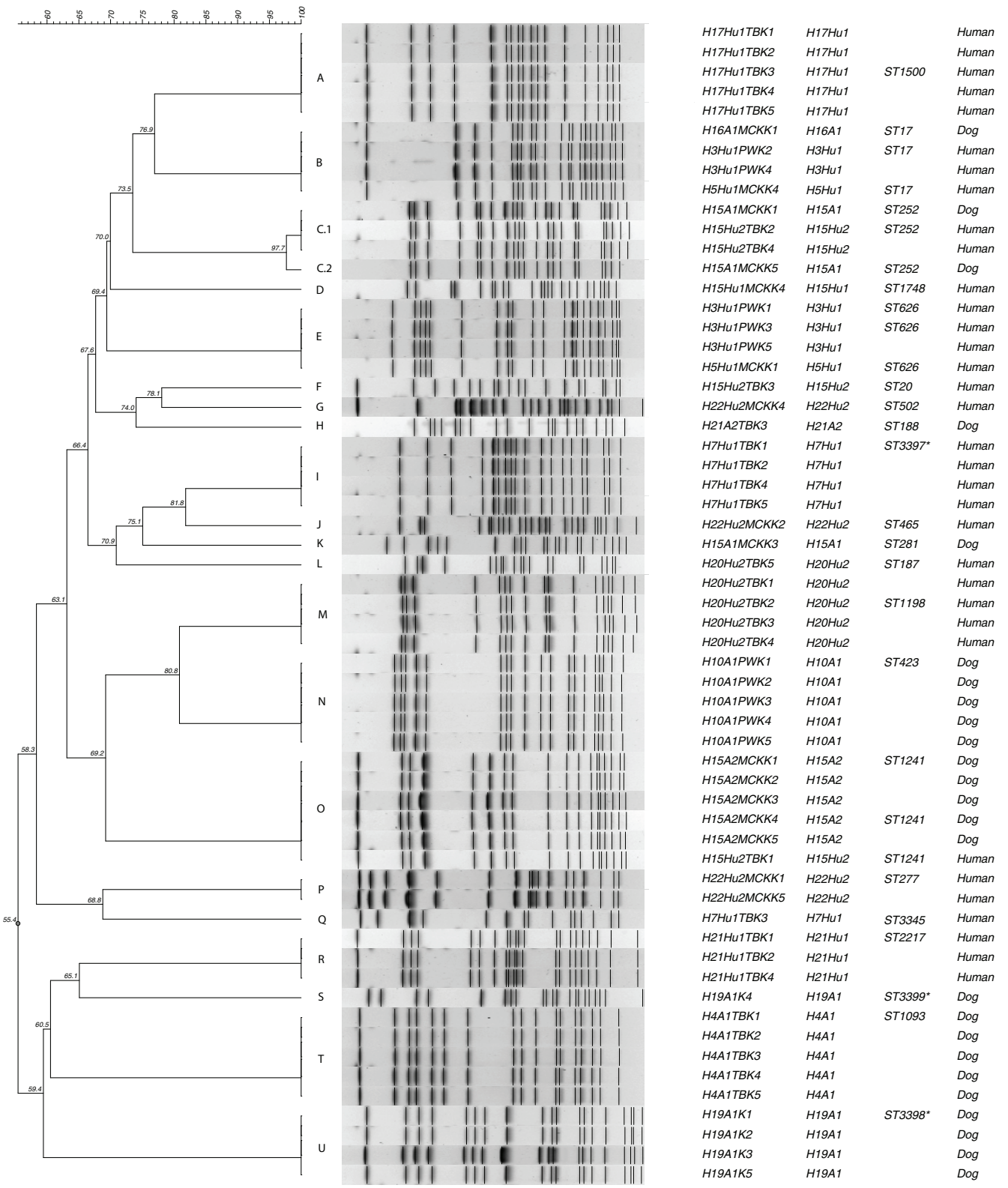


FIG 2 PFGE analysis of commensal *K. pneumoniae* from humans and animals living in close contact. An asterisk (*) indicates a new ST described in this study (ST3397, ST3398, and ST3399).

TABLE 1 Characterization of fecal *K. pneumoniae* strains

Household	Household member	Strain identifier	Pulse type	Sequence type	Clonal complex[b]	Antimicrobial resistance[c]	Virulence genes[d]
H3	Human Hu1	H3Hu1PWK1	E	ST626		None	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kfu*, *allS*
H3	Human Hu1	H3Hu1PWK2	B	ST17	CC11	None	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H4	Dog A1	H4A1TBK1	T	ST1093	CC11	(AK), NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kfu*
H5	Human Hu1	H5Hu1MCKK1	E	ST626		(NIT)	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kfu*, *allS*
H5	Human Hu1	H5Hu1MCKK4	B	ST17	CC11	(CTX), (CN), (AK)	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H7	Human Hu1	H7Hu1TBK1	I	ST3397[a]	CC11	None	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kfu*, *allS*
H7	Human Hu1	H7Hu1TBK3	Q	ST3345	CC11	NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H10	Dog A1	H10A1PWK1	N	ST423	CC11	NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H15	Dog A1	H15A1MCKK1	C.1	ST252	CC11	NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H15	Dog A1	H15A1MCKK3	K	ST281	CC505	NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*, YHPI
H15	Dog A1	H15A1MCKK5	C.2	ST252	CC11	(CN), (AK), NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H15	Dog A2	H15A2MCKK1	O	ST1241		NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kfu*
H15	Human Hu1	H15Hu1MCKK4	D	ST1748	Singleton	NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H15	Human Hu2	H15Hu2TBK1	O	ST1241		NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kfu*
H15	Human Hu2	H15Hu2TBK2	C.1	ST252	CC11	NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H15	Human Hu2	H15Hu2TBK3	F	ST20	CC11	CN, (AK), NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H16	Dog A1	H16A1MCKK1	B	ST17	CC11	(CN), (AK)	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H17	Human Hu1	H17Hu1TBK3	A	ST1500	CC11	NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H19	Dog A1	H19A1K1	U	ST3398[a]	Singleton	(CN), NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kfu*
H19	Dog A1	H19A1K4	S	ST3399[a]	Singleton	(CN), NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kfu*
H20	Human Hu2	H20Hu2TBK2	M	ST1198	CC11	C, NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H20	Human Hu2	H20Hu2TBK5	L	ST187	CC187	(CTX), NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H21	Dog A2	H21A2TBK3	H	ST188	Singleton	(CN)	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kfu*
H21	Human Hu1	H21Hu1TBK1	R	ST2217	Singleton	(CN), NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kfu*
H22	Human Hu2	H22Hu2MCKK1	P	ST277	CC11	(CN), NIT	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H22	Human Hu2	H22Hu2MCKK2	J	ST465	CC11	TE	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*
H22	Human Hu2	H22Hu2MCKK4	G	ST502	CC502	None	*fimH*-1, *mrkD*, *ycfM*, *entB*, *kpn*

[a]ST3397, ST3398, and ST3399 are new STs described in this study.
[b]Clonal complexes (CC) were assigned based on the predicted founder ST based on a population snapshot by eBURST analysis of all *K. pneumoniae* sequence types known until 31 August 2018.
[c]Intermediate resistance is indicated in parentheses. AK, amikacin; C, chloramphenicol; CN, gentamicin; CTX, cefotaxime; NIT, nitrofurantoin; TE, tetracycline.
[d]*fimH*-1, type 1 adhesin; *mrkD*, type 3 adhesin; *kpn*, FimH-like adhesin; *ycfM*, outer membrane lipoprotein; *entB*, enterobactin; *kfu*, iron transporter with phosphotransferase function; YHPI, yersiniabactin high-pathogenicity island; *allS*, allantoin metabolism-associated gene.

(Fig. 1 and 2). The colonized human participants from households H3 and H5 and the human living in close contact (H16Hu1) with the colonized dog H16A1 share the same workplace and thus are epidemiologically related. Notably, the colonized dog H16A1 does not visit the workplace of human H16Hu1.

Overall, within-household human-animal sharing of *K. pneumoniae* strains occurred in 5.5% ($n = 1/18$) of all included households and in 8.3% ($n = 1/12$) of positive households. Considering the positive participants, there were a total of 20 human-animal pairs where potential within-household sharing was possible. Based on PFGE results, 10% ($n = 2/20$) of these human-animal potential pairs shared undistinguishable *K. pneumoniae* strains. Although several households included multiple human participants, none of the colonized humans shared *K. pneumoniae* strains with the coliving humans ($n = 5$ households) (Fig. 1). The same was true for animals living with colonized dogs ($n = 3$ households) (Fig. 1).

Overall, 27 *K. pneumoniae* strains were typed by MLST and characterized for antimicrobial resistance and for the presence of virulence genes.

A total of 21 STs, corresponding to the different PFGE pulse types, were detected, thus revealing high *K. pneumoniae* population diversity in colonized dogs and humans (Table 1). Three novel *K. pneumoniae* STs were described, namely ST3397 to ST3399. In $eBURST_{v3}$ analysis, the novel ST3398 and ST3399 strains isolated from a dog were found to be singletons. The *K. pneumoniae* ST3397 strain isolated from a human was a double-locus variant from ST65.

Most *K. pneumoniae* strains were susceptible to the tested antimicrobials. The only exception was the antimicrobial nitrofurantoin, against which 70.4% ($n = 19/27$) of strains were not susceptible. Furthermore, several strains were intermediately resistant against gentamicin and/or amikacin (Table 1). The two *K. pneumoniae* strains with

intermediate resistance to cefotaxime were negative for all of the beta-lactamase genes tested except bla_{SHV}.

Two main virulence genotypes were detected, namely *fimH-1 mrkD ycfM entB kfu* and *fimH-1 mrkD ycfM entB kpn* (Table 1). The *allS* gene was only detected in *K. pneumoniae* strains from humans belonging to ST626 and to novel ST3397. The yersiniabactin high-pathogenicity island was present in one strain from a dog belonging to ST281 (Table 1). All *K. pneumoniae* isolates were negative for *rpmA*, *iutA*, *iroN*, and *traT* genes and positive for *ompK35* and *ompK36*. Moreover, only one strain lacked *oqxAB* genes according to PCR.

The *K. pneumoniae* strains from the participants sharing identical pulse type/STs also shared the same virulence genotype. However, the antimicrobial resistance phenotype was not always similar (pulse types E and B) (Table 1).

The *K. pneumoniae* strains shared between the H15 human/dog pairs belonged to ST252 (pulse type C.1) and ST1241 (pulse type O) and also had an identical antimicrobial resistance phenotype and an identical virulence genotype (Table 1). Both household H15 human participants reported that they allowed all the household dogs ($n = 3$) to lick their faces and sleep in their beds. No difference in dog-dog or dog-human behavior was noted regarding the three dogs living together. Interestingly, the dog H15A1 was colonized by two variants (pulse type C.1 and C.2) of the same *K. pneumoniae* pulse type (Fig. 1, Table 1). The *K. pneumoniae* strains (pulse type B) shared between the two humans and one dog from different households all belonged to ST17; however, these strains presented different antimicrobial resistance phenotypes (Table 1).

Some of the fecal *K. pneumoniae* strains, mostly from humans, showed a ≥80% similarity to strains from humans with UTI (Fig. 3). Of note, dog H10A1 was colonized with a *K. pneumoniae* strain that was closely related (92.3% similarity) to a clinical ST423 CTX-M-15-producing *K. pneumoniae* strain (PC25/15B) from a human (Fig. 3). The dog and human from household H10 did not have prior clinical history of UTI, were not under antimicrobial treatment in the last year, and had no contact with the hospital environment.

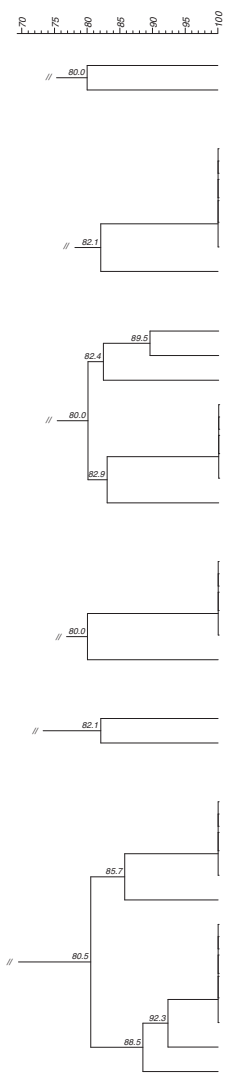
DISCUSSION

To our best knowledge, this is the first report of the fecal colonization and sharing of *K. pneumoniae* clonal lineages between healthy humans and dogs living in close contact.

The high *K. pneumoniae* population diversity detected in this study is in line with that in previous reports (7, 8, 34). There are several *K. pneumoniae* STs disseminated worldwide, including in Portugal, that are considered to be high-risk clonal lineages or are recognized as important international outbreak clones (35, 36). Considering that the gut is a reservoir of pathogenic *Enterobacteriaceae* (1), it is interesting to notice that only the high-risk ST17 and the international outbreak ST20 *K. pneumoniae* clonal lineages were detected in this study. The *K. pneumoniae* high-risk clonal lineages are frequently ESBL and carbapenemase producers (36). The fact that most of the fecal *K. pneumoniae* strains from this study were susceptible to beta-lactams may explain the low frequency of high-risk clonal lineages detected.

K. pneumoniae ST15, which is a high-risk clonal lineage, seems to predominate among clinical CTX-M-15-producing strains from companion animals from several countries (4, 7–9). In a previous study conducted in dogs and cats with UTI from Portugal, most uropathogenic *K. pneumoniae* isolates also belonged to ST15 (9). The absence of colonized dogs with *K. pneumoniae* ST15 was therefore a surprise.

Several studies have reported the colonization and sharing of *E. coli* strains between companion animals and humans (13–18). To our best knowledge, data on *K. pneumoniae* is still lacking. The use of PFGE over whole-genome sequencing (WGS) could be considered a limitation of the current study, since the latter is more discriminatory and is necessary to definitely ascertain the similarity of bacterial strains. However, previous studies on *K. pneumoniae* outbreaks have found that WGS-based phylogeny is consis-



PFGE xbal restriction patterns	Strain code	Participant	Species	Source	UTI vs Helthy	Year	ST
	GTBA713	-	Human	Urine	UTI-C	2014	-
	H15Hu1MCKK4	H15Hu1	Human	Feaces	Healthy	2016	ST1748
	H17Hu1TBK1	H17Hu1	Human	Feaces	Healthy	2016	-
	H17Hu1TBK2	H17Hu1	Human	Feaces	Healthy	2016	-
	H17Hu1TBK3	H17Hu1	Human	Feaces	Healthy	2016	ST1500
	H17Hu1TBK4	H17Hu1	Human	Feaces	Healthy	2016	-
	H17Hu1TBK5	H17Hu1	Human	Feaces	Healthy	2016	-
	PC108/07B	-	Human	Urine	UTI-H	2007	-
	GTBC269	-	Human	Urine	UTI-C	2014	-
	GTCL480	-	Human	Urine	UTI-C	2014	-
	GTLV2220	-	Human	Urine	UTI-C	2014	-
	H16A1MCKK1	H16A1	Dog	Feaces	Healthy	2016	ST17
	H3Hu1PWK2	H3Hu1	Human	Feaces	Healthy	2016	ST17
	H3Hu1PWK4	H3Hu1	Human	Feaces	Healthy	2016	-
	H5Hu1MCKK4	H5Hu1	Human	Feaces	Healthy	2016	ST17
	GTET170	-	Human	Urine	UTI-C	2014	ST6
	H3Hu1PWK1	H3Hu1	Human	Feaces	Healthy	2016	ST626
	H3Hu1PWK3	H3Hu1	Human	Feaces	Healthy	2016	ST626
	H3Hu1PWK5	H3Hu1	Human	Feaces	Healthy	2016	-
	H5Hu1MCKK1	H5Hu1	Human	Feaces	Healthy	2016	ST626
	PC091/09B	-	Human	Urine	UTI-H	2009	-
	GTLA4944	-	Human	Urine	UTI-C	2014	-
	H15A1MCKK3	H15A1	Dog	Feaces	Healthy	2016	ST281
	H20Hu2TBK1	H20Hu2	Human	Feaces	Healthy	2016	-
	H20Hu2TBK2	H20Hu2	Human	Feaces	Healthy	2016	ST1198
	H20Hu2TBK3	H20Hu2	Human	Feaces	Healthy	2016	-
	H20Hu2TBK4	H20Hu2	Human	Feaces	Healthy	2016	-
	GTVIM3185	-	Human	Urine	UTI-C	2014	-
	H10A1PWK1	H10A1	Dog	Feaces	Healthy	2016	ST423
	H10A1PWK2	H10A1	Dog	Feaces	Healthy	2016	-
	H10A1PWK3	H10A1	Dog	Feaces	Healthy	2016	-
	H10A1PWK4	H10A1	Dog	Feaces	Healthy	2016	-
	H10A1PWK5	H10A1	Dog	Feaces	Healthy	2016	-
	PC25/15B	-	Human	Urine	UTI-H	2015	ST423
	GTML16821	-	Human	Urine	UTI-C	2014	-

FIG 3 Clusters of *K. pneumoniae* isolated from clinical UTI and from fecal samples of human and companion animal origin. UTI-C, community patient with UTI; UTI-H, hospitalized patient with UTI.

tent with the PFGE and MLST data combined, especially in strains differing in less than 3 bands (37, 38). Therefore, the detection of dogs and a human living in the same household (H15) colonized by *K. pneumoniae* strains belonging to the same ST, with undistinguishable PFGE restriction patterns, with an identical antimicrobial resistance phenotype and an identical virulence genotype is strongly suggestive of human-dog *K. pneumoniae* sharing.

Household H15 is also remarkable because although 2 humans and 2 dogs were colonized by *K. pneumoniae*, within-household sharing was only detected between human-dog pairs. The absence of human-human or dog-dog sharing in this study is likely related to the low number of households with multiple colonized humans or dogs. The *K. pneumoniae* ST252 clonal lineage shared between the human H15Hu2 and dog H15A1 has been previously detected in fecal samples from hospitalized patients and long-term-care facility residents from Portugal (39, 40). This could suggest that colonization had human origin. However, the higher *K. pneumoniae* ST252 and ST1241 fecal burden detected in these dogs could point to dog-to-human transmission. Allowing the dog to lick the face has been suggested as a risk factor for dog-human *E. coli* sharing (18). However, in household H15 this was not a determining factor. A common source of *K. pneumoniae* acquisition should also be hypothesized, but since

the three dogs had a common living environment and behaviors, it would be likely that the three dogs would be colonized by the same strains. *K. pneumoniae* is known to spread easily (1); therefore, additional studies are necessary to clarify its routes of human-dog dissemination.

The two humans and one dog living in different households that were colonized by PFGE-undistinguishable *K. pneumoniae* ST17 strains were epidemiologically related, and therefore the *K. pneumoniae* transmission could have occurred through direct (human-human) or indirect (human-dog) contact. However, it should be noted that the antimicrobial resistance phenotypes of these *K. pneumoniae* ST17 strains differed. Thus, since the *K. pneumoniae* ST17 clonal lineage is disseminated worldwide (36), the circulation of this strain in the community and the colonization of these participants through unrelated sources is the likely explanation. For instance, retail meat has been pointed to as a potential source of uropathogenic *K. pneumoniae* to humans (41). The detection of a high-risk *K. pneumoniae* clonal lineage colonizing a healthy dog highlights its possible role as a reservoir. Furthermore, other *K. pneumoniae* clonal lineages that were detected in dogs, namely ST188, ST252, ST281, ST423, and ST1093, have also been previously implicated in human infections (39, 41–44).

The colonization of humans and dogs by *K. pneumoniae* was equally high (~38%), and 10% of the potential within-household human-animal pairs shared *K. pneumoniae* strains undistinguishable by PFGE. Since this study relied on standard culture procedures, *K. pneumoniae* colonization and sharing could be underestimated due to the overgrowth of other *Enterobacteriaceae* species. Nevertheless, a previous study from India has reported that only 26% of the healthy dogs were colonized by *K. pneumoniae* (45). Considering that 61.5% of participants were colonized by multiple strains, it can be speculated that the PFGE typing of a higher number of colonies per sample could be advantageous in future studies to detect additional sharing pairs. The nasopharynx is also a *K. pneumoniae* colonization site (2). In retrospect, we find that the study of nasopharyngeal colonization could have undisclosed further epidemiological links between colonized dogs and humans. The absence of colonized cats in this study may be related to the number of cats tested, since infections caused by *K. pneumoniae* have been previously reported in cats (9).

The frequency of *K. pneumoniae* virulence genes agrees with previously published data (28, 29, 34). The absence or low frequency of virulence genes associated with higher *K. pneumoniae* invasiveness (34) is a positive outcome from this study. The first hypervirulent *K. pneumoniae* ST23 isolate detected in Portugal was only recently described in a human patient (46). Therefore, the absence of hypervirulent *K. pneumoniae* clonal lineages in this study was expected.

According to the annual report of the European Antimicrobial Resistance Surveillance Network, Portugal is among the countries with a higher frequency of resistance to third-generation cephalosporins, carbapenems, and fluoroquinolones in invasive *K. pneumoniae* strains (6). Additionally, a high frequency of fecal colonization by ESBL/AmpC-producing *K. pneumoniae* has been reported in long-term-care facility residents from Portugal (40). The high susceptibility and lack of multidrug-resistant *K. pneumoniae* isolates in the present study is, therefore, considered a positive finding. Nevertheless, these results may be a consequence of the study design due to the limited sample size and because it relied on healthy humans and animals without reported infections or antimicrobial use in the prior month.

Another interesting finding from this study was the detection of one healthy dog (H10A1) colonized by a *K. pneumoniae* ST423 strain that was 92.3% similar by PFGE to one strain isolated from a human with UTI. The use of WGS is warranted to fully disclose the relatedness of these strains; nevertheless, this finding should not be neglected. *K. pneumoniae* can cause other important infections, such as pneumonia (1, 34). For this reason, future comparative studies should include *K. pneumoniae* strains from other clinical origins to further understand the role of dogs as reservoirs of pathogenic *K. pneumoniae*. In fact, the *K. pneumoniae* clonal lineages detected in dogs from this study

have been previously isolated from several types of human infections besides UTI (39, 42–44).

To conclude, this study presents novel epidemiological data regarding *K. pneumoniae* colonization and suggests that healthy humans and dogs may share similar *K. pneumoniae* clonal lineages. The role of dogs as reservoirs of *K. pneumoniae* clonal lineages previously described in human infections is noteworthy, even though those strains were neither multidrug resistant nor hypervirulent. Some questions remain to be answered regarding the routes of transmission and persistence of *K. pneumoniae* colonization over time in coliving humans and dogs. Future studies using longitudinal designs should be conducted to clarify these issues regarding infected and healthy companion animals. Meanwhile, good hygiene practices and proper fecal disposable should be advised to dog caretakers to minimize the chances of direct or indirect *K. pneumoniae* interspecies transmission.

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